Notice of Meeting and Call for Abstracts

30th PLANT DEVELOPMENT WORKSHOP

Saturday, April 8, 1995
Davis Center
University of Waterloo
Hosted by Dr. Carol Peterson

Preliminary Program:

Registration and coffee..... 9:00 AM

First keynote address.....10:00 AM
Dr. Barry Tomlinson, Harvard University
THE CONIFEROUS SEED CONE - DEVELOPMENTAL ASPECTS

Contributed papers and discussion..... 10:30 AM

Coffee break..... 11:00 - 11:15 AM

Contributed papers and discussion..... 11:15 AM

Lunch and Posters..... 12:15 - 2:15 PM

Second keynote address..... 2:15 PM
Dr. John Thompson, University of Waterloo
TOPIC - DETERIOSOMES

Contributed papers and discussion..... 3:15 PM

Wine and cheese reception..... 4:00 PM

Please return the attached abstract form by **March 10, 1995**, indicating the number attending. Further information will be mailed to respondents the week of March 27.

Abstract Form

30th PLANT DEVELOPMENT WORKSHOP

Saturday, April ~~8~~ 27 1996, 1995
Davis Center
University of Waterloo

Please fit abstract in a box 15 X 10 cm. Do not overlap the box.

Return abstract by **March 10, 1995** to: April 5/96

Dr. Carol Peterson
Department of Biology
University of Waterloo
Waterloo, Ontario N2L 3G1

Paper ☐ Poster ☐ People attending _____

30th PLANT DEVELOPMENT WORKSHOP

Program and Abstracts

Department of Biology
University of Waterloo

Davis Center
Saturday, April 8, 1995

Cover figure courtesy of Dr. J. Semple; used with permission.

30th Plant Development Workshop

April 8, 1995
University of Waterloo
Davis Center - Room 1302

RCA Cyplan
PCR.

8:45 - 9:30 AM Registration and coffee, Davis Center - Room 1301

Chair of Morning Sessions: Judy Canne-Hilliker, University of Guelph

First keynote address - Room 1302

9:30 **Seed Cone Development in Conifers**
P. BARRY TOMLINSON, Harvard University

Contributed papers and discussion ..... 10:00 - 10:45 AM (Room 1302)

10:00 **Genetic Control of Cone Development in Black Spruce**
SHARON REGAN, CHANTAL COTE, DON STEWART, CHRIS KAUFFELDT, WHYNN BOSNICH, BOB RUTLEDGE, Petawawa National Forestry Institute

10:15 **Characterization of Embryogenesis-related Gene Products in Alfalfa (*Medicago sativa* L.)**
RANDAL W. GIROUX, PETER PAULS, University of Guelph

10:30 **The Pollen-tube Pathway and the Role of the Obturator in *Crataegus***
NINA J. CELOTTI[1], T. A. DICKINSON[2*], A. LIN[3], Queen's University[1] Royal Ontario Museum[2], and University of Toronto[2,3]

10:45 - 11:00 Coffee Break

11:00 **Leaf Morphogenesis and Growth in *Cyperus eragrostis* a C3 Sedge**
CONNIE L. SOROS-POTTRUFF, NANCY G. DENGLER, University of Toronto

11:15 **Phenotypic Plasticity in Leaves of Four Species of Arctic *Festuca***
N. RAMESAR-FORTNER[1], S. G. AIKEN[2], N. G. DENGLER[1] University of Toronto[1], and Canadian Museum of Nature[2]

11:30 **Developments in Epi-illumination Light Microscopy and Macrophotography in Morphology**
W. ALAN CHARLTON[1], ALASTAIR D. MACDONALD[2], USHER POSLUSZNY[3], University of Manchester[1], Lakehead University[2], and University of Guelph[3]

11:45 **Use of Acid Digestion to Study Endodermis and Hypodermis Development and Structure in Roots of Selected Wetland Plants**
JAMES L. SEAGO JR.[1], CAROL A. PETERSON[2], DARYL E. ENSTONE[2], State University of New York[1], and University of Waterloo[2]

12:00 **Exodermal Casparian Bands and Band Plasmolysis in Corn Roots,**
DARYL E. ENSTONE, CAROL A. PETERSON*, University of Waterloo

12:15 - 2:15 PM Lunch and Posters - Room 1301

POSTERS

1. **A Three-dimensional Reconstruction of the Male Germ Unit in *Asclepias tuberosa***
KAREN K. LEE, TAMMY L. SAGE, University of Toronto

2. **Flow Cytometric Characterization of Embryogenesis in *Brassica napus* Microspore Cultures**
DEREK SCHULZE, PETER PAULS, University of Guelph

3. **The Developmental Plasticity of *Ranunculus flabellaris* in response to aerial and aquatic environments**
NADIA C. BRUNI[1], JANE P. YOUNG[2], NANCY G. DENGLER[1], University of Toronto[1], and University of Northern British Columbia[2]

4. **Orientation of Cellulose Fibers in Pea Tendrils**
JEAN M. GERRATH[1], RICHARD CÔTÉ[2], University of Northern Iowa[1], University of Guelph[1,2]

5. **Effect of Viral Infection on Cell Wall Integrity**
FAUZIA SIDDIQ, SANDRA A. KOFALVI, ANNETTE NASSUTH, University of Guelph

6. **Examination of the Phenolic Compounds in Concorde and Fredrick Wheat and Barley spp.**
C. L. MILES, A. M. ZOBEL, Trent University

7. **Response of Flavonoids to Cadmium Treatment**
B. F. PRIMEAU, A. M. ZOBEL, Trent University

8. **Regulation of Anthocyanin Biosynthesis in Pea, *Pisum sativum***
ANNE UIMARI, JUDITH STROMMER, University of Guelph

9. **The Effects of UV Radiation and Acid Spray on the Production of Some Secondary Metabolites in *Daucus carota* and *Ruta graveolens***
C. BATES, A. M. ZOBEL, Trent University

10. **The Biochemical Changes in Plant Cells Due to Toxic Concentrations of Silver**
TERESA SWITZER, ALICJA M. ZOBEL, Trent University

11. **The Influence of Tea and Echinacea on the Chemistry of the Mitochondria Treated with Olive Oil**
FOO-LIM YEH, ALICJA ZOBEL, Trent University

12. **Cellular Effects of Roundup®**
C. A. SWACKHAMMER, A. M. ZOBEL, Trent University

Chair of afternoon sessions: John Lott, McMaster University

Contributed papers and discussion ..... 2:15 - 3:00 PM (Room 1302)

2:15 **Root Anatomy of Container-grown *Thuja occidentalis* (Eastern White Cedar)**
J. DOUBT, D. LARSON, R. L. PETERSON, University of Guelph

2:30 **Mycorrhizal Colonization and Growth of Eastern White Pine (*Pinus strobus* L.) Seedlings at a Bare-root Nursery in Southern Ontario**
MARGOT KRONICK, R. LARRY PETERSON, University of Guelph

2:45 **The Interface Between Fungal Hyphae and Orchid Protocorm Cells**
L. PETERSON,[1], P. BONFANTE[2], University of Guelph[1], and Università di Torino[2]

Second keynote address

3:00 **Impairment of Catabolite Blebbing from Membranes Engenders Cellular Dysfunction In Senescing Tissue**
JOHN E. THOMPSON, University of Waterloo

3:30 - 3:45 Coffee Break

Contributed papers and discussion ..... 3:45 - 5:00 PM (Room 1302)

3:45 **Excitation Pressure and Redox Control: Shedding Some Light on Low Temperature Development in Cold Tolerant Plants**
GORDON R. GRAY[1], LOUIS-PIERRE CHAUVIN[2], FATHEY SARHAN[2], NORMAN P. A. HUNER[1], University of Western Ontario[1], and Université du Québec à Montréal[2]

4:00 **Natural Growth Retardants and Stress Management**
S. HAYWARD, A. M. ZOBEL, Trent University

4:15 **Phenolic Induction Upon Ultraviolet Treatment in Austrian Pine**
KEITH E. GALE, ALICJA M. ZOBEL, Trent University

4:30 **Extrusion of UV Absorbing Phenolic Compounds in *Acer* Under Stress**
J. M. LYNCH, A. M. ZOBEL, Trent University

4:45 **Soybean Seed-coat Extracts and Arrest of Microbial Growth**
NANCY SCHMITZ, ROBERT B. VAN HUYSTEE, University of Western Ontario

5:00 PM Wine and cheese reception - Room 1301

ABSTRACTS FOR ORAL PRESENTATIONS

9:30 AM

Seed cone development in conifers. P. B. Tomlinson, Harvard Forest, Petersham, MA 01366 U.S.A. Can development be useful in evolutionary interpretation? It should be, since evolutionary change is the result of (gradual ?, abrupt?) changes in developmental processes. Unfortunately, the fossil record rarely provides developmental stages of organs or organ complexes. One might infer developmental features from comparison of extant with extinct groups, provided development is known in the former and homologies can be established, perhaps based on part-for-part equivalence. The coniferous seed cone provides an opportunity because the origin of its ovule-bearing parts has been suggested by Florin ("the Florin model"), and the seed cone of modern conifers is very diverse. Developmental evidence, however, shows that the putative compound structure of the coniferous cone, implied in the Florin model, is lost from many modern groups so that cones are (or have become) simple. Much of this simplification (reduction?) involves specialization for pollination, protection and dispersal (i.e. functional considerations need to be introduced). The seed cone of conifers thus provides a wonderful opportunity for the deployment of popular h-words, like: - heterochrony, heterotropy, homeosis and homology, but is, more importantly, illustrative of the relation between development, function and evolution.

10:00 AM

Genetic Control of Cone Development in Black Spruce

Sharon Regan, Chantal Cote, Don Stewart, Chris Kauffeldt, Whynn Bosnich, and Bob Rutledge, Petawawa National Forestry Institute, Natural Resources Canada, Chalk River, Ontario

We are interested in understanding the molecular mechanisms which control cone development in conifers. Although little is known about the molecular factors involved in this process, several homeotic genes which determine floral meristem and organ identity have been characterized in angiosperms such as *Arabidopsis* and *Antirrhinum*. Many of these genes contain a highly-conserved domain known as the MADS box, likely involved in binding DNA in a sequence-specific manner. Using PCR cloning, we have estimated that black spruce (*Picea mariana*) contains a large family of MADS box-related genes which may be divided into 10 to 15 subfamilies. Several cDNA clones expressed in male and female cones have now been isolated and are being further characterized. Of particular interest is a clone, called SMADS42, which has extensive sequence similarity with the MADS-box protein agamous from *Arabidopsis*. Since agamous is partially responsible for determining floral organ identity in *Arabidopsis*, we have initiated several experiments to determine whether SMADS42 serves a similar function in spruce.

10:15 AM **Characterization of Embryogenesis-related Gene Products in Alfalfa (*Medicago sativa* L.).** Randal W. Giroux and K. Peter Pauls, Crop Science Department, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Plant tissue cultures are useful for studying mechanisms of plant development. In particular, cellular and molecular analyses of somatic embryogenesis can provide information about the earliest stages of plant development. Differences in the abiltity of embryogenic and non-embryogenic genotypes of alfalfa to regenerate in tissue culture should also be accompanied by differences in gene expression. Messanger RNAs from embryogenic and non-embryogenic alfalfa cultures were used to differentially screen a cDNA library prepared from embryogenic cell masses, to isolate clones encoding transcripts found in early-stage embryos. Three alfalfa somatic embryogenesis-specific transcripts (*ASET1*, *ASET2* and *ASET3*) were identified from this screen. These clones only hybridized to Northern blots from embryogenic cultures. DNA sequencing revealed that *ASET1* was a partial transcript showing weak homology to ATPase sequences and *ASET2* was a complete transcript coding for a protein with several membrane-spanning domains and a potential phosphorylation site. In addition, the *ASET2* cDNA had a long 5' region that contained two upstream reading frames (URFs) which coded for 30 and 6 amino acid peptides. These results support the hypothesis that somatic embryogenesis in alfalfa is determined by the expression of specific developmental genes.

10:30 AM CELOTTI, NINA J.[1], DICKINSON, T. A.[2], and A. LIN[3]. **The pollen-tube pathway and the role of the obturator in Crataegus.** [1]Biology Department, Queen's University; [2]Botany Departments, Royal Ontario Museum and University of Toronto; and [3]Zoology Department, University of Toronto.

The pollen–tube pathway and the role of the obturator were investigated in native Crataegus punctata Jacq. and introduced C. monogyna Jacq. growing together at a site near Toronto. Both taxa are known to be diploid in Ontario, and to exhibit self–sterility. Compatible and incompatible pollinations were carried out, and then harvested after successive time intervals. Harvested flowers were dissected and examined by means of epifluorescence microscopy (staining with Aniline Blue to localize pollen tube callose), SEM, and energy–dispersive X–ray microanalysis (to localize calcium). We observed significant differences in the distribution and behavior of pollen tubes between successive portions of the pollen–tube pathway and between self– and cross–pollinated flowers; these differences parallelled the contrast in seed–set between the two treatments. Calcium appeared to be concentrated in the obturator. Altogether, the results obtained support a role for the obturator in guiding pollen tubes to the micropyle of one of the two ovules in each locule. The data obtained provide somewhat weaker evidence for the role of calcium in this guidance.

11:00 AM

Connie L. Soros-Pottruff* and Nancy G. Dengler. Department of Botany, University of Toronto. Toronto, Ontario. - Leaf morphogenesis and growth in *Cyperus eragrostis* a C3 sedge.

The specific goal of this study is the identification of the extension zone, and zones of formative cell division, elongation and differentiation in a member of the Cyperaceae, *Cyperus eragrostis*. Leaf lengths were measured over a time span of 40 days and developing shoots were marked near the base of the stem to locate the zone of leaf extension growth. A series of fine insect pins mounted 2mm apart was pushed through the base of the shoot, the leaves were harvested after 48 hours and the distances that the pin punctures had travelled since the initial puncturing were measured. The location of the zones of most frequent cell division and elongation were assessed by examining epidermal cell size and shape in cleared leaves by light microscopy. Leaves are initiated about every 2 days and successive leaves have similar rates and duration of growth. The zone of leaf elongation is within the bottom 8mm of the leaf. Cell length measurements also revealed a short basal elongation zone with 95% of the leaf consisting of elongated cells. Serial cross sections indicated a tristichous phyllotaxis. Leaves of members of the Cyperaceae have a relatively simple pattern of growth in that only the cells in the basal meristematic zone are dividing and cells become progressively older with increasing distance from the meristem. A young leaf can be divided into three zones: 1) the meristematic zone at the base where cell divisions occur, 2) a zone above the base where cells are elongating but no longer dividing and 3) a zone where cells have reached a final length. This pattern of leaf development is almost equivalent to that found in the closely related Poaceae.

11:15 AM

PHENOTYPIC PLASTICITY IN LEAVES OF FOUR SPECIES OF ARCTIC FESTUCA. N. Ramesar-Fortner[1], S. G. Aiken[2], N. G. Dengler[1].
[1] Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1.
[2] Research Division, Canadian Museum of Nature, Ottawa, Ontario K1P 6P4.

Leaf phenotypic plasticity of 13 morphological, anatomical and growth traits was investigated using four species of arctic Festuca (F. baffinensis, F. brachyphylla, F. edlundiae, and F. hyperborea). Plants collected around 78° N in the Canadian Arctic Archipelago were grown for 10 weeks at the University of Toronto in growth chambers in continuous light, under four regimes of temperature and moisture. Significant differences were found between leaves at the time of field collection and leaves of the same plant at the end of the experiment in: a) leaf blade length, b) surface vestiture, both in trichome density and angle of the trichomes to the blade surface, and c) characters seen in leaf cross-sections: blade width, rib thickness, and inter-rib thickness. The four species responded similarly to the experimental conditions, but more variability was observed among plants of F. hyperborea (among-treatment variability was significant in five of twelve traits at $P < 0.05$). In a two-way analysis of variance of leaf traits at the end of the experiment, leaf blade width, rib thickness, and number of sclerenchyma strands showed a more significant species effect than treatment effect, suggesting that these are taxonomically useful traits. Trichome density was the only characteristic for which species showed differing patterns of response, with a unique pattern of response for F. edlundiae. This and certain growth traits support the taxonomic status of this new species.

11:30 AM

W. ALAN CHARLTON*, ALASTAIR D. MACDONALD AND USHER POSLUSZNY, Biological Sciences, University of Manchester, Manchester M13 9PT, U.K., Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1 and Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1. - **Developments in epi-illumination light microscopy and macrophotography in morphology.**

We present a review of methods available for staining shoot apices and small plant parts for epi-illumination light microscopy and macrophotography. Shoot apices and leaf primordia have a cuticle which is sufficiently well-developed to impede the entry of many stains so that the choice of stains that can be used is limited and therefore procedures are quite empirical. Nigrosin, fast green, acid fuchsin are the most successful stains so far in this respect. Where cut surfaces are exposed more specific stains can be used, such as alcian blue. We include the use of double-staining techniques, particularly for macrophotography, and the use of contrast-enhancing filters in the light path.

11:45 AM

Seago, James L. Jr., *, Carol A. Peterson, and Daryl E. Enstone. Department of Biology, SUNY, Oswego, NY 13126 and Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1. - Use of acid digestion to study endodermis and hypodermis development and structure in roots of selected wetland plants.

To assist in the determination of the presence and level of maturation of cells with Casparian bands and suberin lamellae, we employed acid digestion procedures using concentrated H_2SO_4. Adventitious roots of Butomus umbellatus, Nymphoides cordatum, Pontederia cordata, Typha angustifolia, T. glauca, and T. latifolia were observed. Typha species and Butomus exhibited very similar endodermal and hypodermal cell walls which resisted acid digestion. The single-layered endodermis had somewhat wavy longitudinal walls, and the hypodermis was a multi-layered zone in which its exodermis had somewhat wavy to wavy longitudinal walls. Pontederia had a single layer of endodermis and a single layer of exodermis in its hypodermis; the latter was not very thick-walled or wavy. Roots of Nymphoides had an endodermis characterized by extremely wavy walls and an exodermis with mostly non-wavy walls in its large cells. Pro-endodermal and pro-exodermal cells within the meristem resisted acid digestion for varying lengths of time, varying with their distance behind the apex.

12:00 PM EXODERMAL CASPARIAN BANDS AND BAND PLASMOLYSIS IN CORN ROOTS

Daryl E. Enstone and Carol A. Peterson*
Dept. of Biology, University of Waterloo, Waterloo, ON

Exodermal Casparian band formation in corn roots was examined with fluorescent staining, plasmolytic and acid digestion techniques. Berberine/TBO staining revealed that the number of exodermal cells with Casparian bands increased from almost 0% to 94% over a 3 - 8 d growth period. Cells lacking Casparian bands in 8 d old roots were frequently located adjacent to emerged lateral roots. The exodermal band differed from the endodermal band in three noticeable ways. First, the endodermal band formed virtually simultaneously in all cells, while the exodermal band developed over a period of days, resulting in a patchy appearance. These results were confirmed with acid digestions. Second, the endodermal band progressed in a tidy fashion from a dot-like to a full-wall stage, while the exodermal band usually filled the entire wall from the outset or occasionally exhibited a bipolar appearance in some cells. Third, there were post-acid-digestion structural differences between exodermal and exodermal bands. Regular plasmolysis predominated in 3 - 5 d old tissue, while band plasmolysis peaked in 6 - 7 d old tissue. On average, 56% of band-plasmolysed cells lacked berberine/TBO detectable Casparian bands, supporting the idea that wall-to-membrane linkages involve proteins, not suberin.

2:15 PM J. DOUBT*, D. LARSON and R. L. PETERSON. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1 - Root anatomy of container-grown *Thuja occidentalis* (Eastern White Cedar)

Roots of 8-year-old container-grown *Thuja occidentalis* L. were characterized anatomically using light and fluorescence microscopy. Roots lacked root hairs but were colonized by vesicular-arbuscular mycorrhizal fungi. An irregular, 1-3 layered suberized hypodermis without Casparian strips was present. Lignified, branched phi thickenings developed throughout most of the broad cortex but not in the hypodermis or endodermis. Phi thickenings were especially prominent in the cortical cell layer adjacent to the endodermis. Large cortical intercellular spaces were characteristic of these roots. Starch storage was evident within the stele, which consisted of diarch-tetrarch xylem. Roots showing secondary growth had uniform rows of thick, angular phloem fibers, and long tracheids with bordered pits. Axial xylem and phloem parenchyma contained abundant starch. Anatomical characteristics such as phi thickenings, suberized cell layers, thick cortices, and abundant storage reserves, may help to account for the survival of *T. occidentalis* in a wide range of often stressful habitats.

2:30 PM

MARGOT KRONICK* and R. LARRY PETERSON. Department of Botany, University of Guelph, N1G 2W1 - Mycorrhizal colonization and growth of Eastern White Pine (*Pinus strobus* L.) seedlings at a bare-root nursery in southern Ontario

Pinus strobus L. (white pine) seedlings were excavated from St-Williams nursery beds (near Simcoe, southern Ontario) in order to assess the levels and types of natural mycorrhizal colonization. The overall mycorrhizal colonization was well over 50% despite the presence of root rot fungi in the nursery beds, although there was some variability between compartments. Three ectomycorrhizal fungi (*Hebeloma* sp., *Tuber* sp. and *Thelephora terrestris* Ehrh. Fr.) and one ectendomycorrhizal fungus (E-strain *Complexipes*) were identified using macroscopic and microscopic features. These fungi, with the exception of *T. terrestris*, were also isolated from the *P. strobus* roots. A *Mycelium radicis atrovirens*-type fungus was also isolated but remains unidentified. Fruitbodies of *Hebeloma* sp., *Thelephora terrestris* and *Laccaria* sp. found in the nursery beds confirmed two of the identifications.

2:45 PM

L. PETERSON[1] and P. BONFANTE[2]. [1]Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1. [2]Dipartimento di Biologia Vegatale, Università di Torino, Torino, Italy - The interface between fungal hyphae and orchid protocorm cells

Germinating orchid seeds depend on a fungus to supply nutrients for protocorm development. Colonization of protocorm cells involves the formation of pelotons which are separated from the cell cytoplasm by interfacial matrix material and plasma membrane across which nutrients are exchanged. With time, pelotons collapse and the degenerating hyphae become encased by interfacial matrix material. The objective of this study was to characterize the interface at various stages of development of the symbiosis. Two combinations of symbionts (*Goodyera repens-Ceratobasidium cereale; Spiranthes lacera-Ceratorhiza* sp.) were established *in vitro*. Treatment of LR White embedded sections with antibodies to epitopes of pectin conjugated to colloidal gold showed that pectins were absent in the matrix around healthy pelotons but were present around collapsed pelotons. Likewise, cellobiohydrolase conjugated to colloidal gold showed that a small amount of cellulose was deposited around collapsing hyphae. Aniline blue fluorescence and an antibody against β-1,3 glucans indicated their presence around collapsing pelotons and in hyphal walls.

3:00 PM

IMPAIRMENT OF CATABOLITE BLEBBING FROM MEMBRANES ENGENDERS CELLULAR DYSFUNCTION IN SENESCING TISSUE

J. E. Thompson, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada. N2L 3G1

Studies with several plant tissues including leaves, cotyledons, fruit and petals have indicated that there is a hydrophobic subcompartment within the cell cytosol in the form of lipid-protein particles that originate from membranes. The particles range from 140 to 340 nm in diameter, are formed by blebbing from the membrane surface and appear to serve as a vehicle for removing lipid catabolites from the membrane bilayer. Indeed, the cytosolic particles are enriched in such lipid catabolites as free fatty acids, peroxidized lipids, and sterol and wax esters. Upon formation, these catabolites phase-separate in the membrane bilayer, and the ensuing separated domains may well prompt the blebbing process that leads to lipid-protein particle formation. The cytosolic particles resemble oil bodies, which are also formed by blebbing from membranes, in that they can be isolated by flotation, appear to be circumscribed by a monolayer of phospholipid and contain a dominant ~19 kDa protein that is similar in size to the oleosin of oil bodies. Membrane protein catabolites, including those of the proton ATPase, co-isolate with the cytosolic particles, and the particles also exhibit strong protease activity. Similar lipid-protein particles have been isolated from the stroma of intact chloroplasts. The chloroplast particles are also enriched in free fatty acids and contain catabolites of thylakoid proteins including the CF_1, β and γ subunits of ATPase, cytochrome f and the D2 and 33kDA proteins of photosystem II. The data suggest that blebbing of lipid-protein particles is a mechanism for removing destabilizing catabolites from membrane bilayers that are formed during normal turnover. The blebbing process becomes impaired with advancing senescence, and this correlates with an accumulation of lipid catabolites in senescing membranes and consequent lipid phase separations that lead to loss of membrane function.

3:45 PM

EXCITATION PRESSURE AND REDOX CONTROL: SHEDDING SOME LIGHT ON LOW TEMPERATURE DEVELOPMENT IN COLD TOLERANT PLANTS

Gordon R. Gray, Louis-Pierre Chauvin[1], Fathey Sarhan[1] and Norman P.A. Huner
Department of Plant Sciences, The University of Western Ontario, London, Ontario N6A 5B7 and [1]Département des Sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. 'A', Montréal, Québec H3C 3P8

Cold acclimation refers to a period of growth at low temperature which results in the development of freezing tolerance. Cold acclimation results in many morphological changes, including a characteristic rosette growth habit which is an established marker for freezing tolerance. There are also molecular markers which may be used to select for freezing tolerance, two of which are the cold-induced genes *Wcs19* and *Wcs120*. We have examined winter growth habit and freezing tolerance to determine if the acquisition of these characteristics is a response to temperature and/or light. Alternatively, the development of these attributes may be a response to excitation pressure, reflecting the redox poise of intersystem electron transport and carbon metabolism, which can be modulated by either high-light or low temperature. Utilizing winter and spring cereals, as well as spinach, we demonstrate that excitation pressure can account for morphological differences observed as a result of low temperature growth. However, excitation pressure does not fully explain the development of freezing tolerance when examined through Northern and Western hybridization analyses, and lethal temperature measurements (LT_{50}) based on electrolyte leakage. However, both light and temperature do appear to play independent roles, emphasizing the importance of physiological conditions in the attainment of maximal freezing tolerance.

4:00 PM

Natural Growth Retardants and Stress Management

S. Hayward and A.M. Zobel, Chemistry, Trent University, Peterborough, ON K9H 7B8

Seedlings were exposed to environmental stresses: UV light and ozone gas. Results showed doubling of anthocyanin and flavonoid levels after 36 hrs for seedlings continuously exposed to ozone. Also, oats were soaked in growth retardants (coumarin and xanthotoxin), in order to assess the effects of such compounds on stress management by seedlings. Seedlings were fixed after 6 hrs, 24 hrs, and 54 hrs. Coumarin-soaked seedlings contained higher levels of secondary defence compounds to begin with, and exposure to ozone triggered an increased production of defence compounds in comparison with control and xanthotoxin-soaked seedlings. Transfer of ozonated plants to a UV chamber was beneficial to the plant. Both 366nm and 254nm prolonged the life of the seedling, which opposes the popular theory that UV light is always damaging. Development of oats that produce augmented amounts of these already naturally-occurring secondary metabolites may impart slowed growth and plants which are more readily adaptable to destructive environmental stresses.

4:15 PM

PHENOLIC INDUCTION UPON ULTRAVIOLET TREATMENT IN AUSTRIAN PINE

Keith E. Gale and Alicja M. Zobel, Dept. of Chemistry, Trent University, Peterborough, ON K9J 7B8

Separated branches of Austrian Pine were removed and used to study the induction of phenolic metabolites upon treatment of two wavelengths of UV radiation. It is well known that many plant species can avert or reduce the damaging effect of UV-B radiation by the formation of UV-protecting pigments, as one of its defense systems.

The two wavelengths that the pine was exposed to were 254 nm (artificial) and 366 nm. The 254 nm radiation appears to have caused a greater increase of surface phenolics absorbing in the 330 nm range (i.e. flavones) than did the radiation of 366 nm. The surface phenolics absorbing at 525 nm (anthocyanins) did not appear to be influenced by the ultraviolet radiation in this species.

4:30 PM **EXTRUSION OF UV ABSORBING PHENOLIC COMPOUNDS IN ACER UNDER STRESS**

J.M. Lynch and A.M. Zobel. Department of Chemistry, Trent University. Peterborough, Ontario. K9J 7B8

UV absorbing phenolic compounds play several important roles in the ecology of plants. The protectory role of UV absorbing phenolic compounds against the stress of low temperature and UV radiation was investigated via a comparison of the contrasting genetic expression during ontogenesis visible in leaf colouration each autumn when *Acer saccharum* becomes red and *Acer platanoides* becomes green. Both Acer species had on their surface phenolic compounds that absorb UV irradiation (8-24%). Chemical analysis indicated that all more senescent autumn leaves of both species, in spite of opposite leaf colouration, have an increased concentration of UV absorbing phenolic compounds than less senescent ones. Both the green and red leaves of *Acer saccharum* possessed more UV absorbing phenolic compounds than leaves of *Acer platanoides*. The results suggested that the biosynthesis of UV absorbing phenolic compounds is stress induced (UV and freezing temperatures) and is a non-specific defensive response.

4:45 PM Soybean seed-coat extracts and arrest of microbial growth

Nancy Schmitz and Robert B. van Huystee, Department of Plant Sciences, University of Western Ontario, London, Ontario, N6A 5B7.

A correlation between peroxidase activity and disease resistance has been reported. The peroxidase activity has been shown to occur in the our glass cells of the epidermis in the seed-coat of soybean (Plant Physiol. 103: 1061, 1993). Seed-coats from resistant and susceptible soybeans have been extracted and the effect of the extract on the growth of several pathogens has been determined. The ultimate aim is to isolate either peroxidase or any other seed-coat compound that causes arrest of growth.

ABSTRACTS FOR POSTERS

1. **A Three-Dimensional Reconstruction of the Male Germ Unit in *Asclepias tuberosa*.** LEE, KAREN K. and TAMMY L. SAGE, Botany Dept., University of Toronto, Toronto, Ontario M5S 3B2

The two sperm cells and the vegetative nucleus (collectively referred to as the male germ unit - MGU) of *Asclepias tuberosa* were qualitatively and quantitatively characterized using three-dimensional reconstruction and morphometric analysis of fifty pollen grains. The two sperm cells are elongate in shape while the vegetative nucleus is an irregular, multi-lobed oval. Sperm cells are frequently in physical contact with each other as well as the vegetative nucleus; in some cases, the vegetative nucleus contained sperm cell cytoplasmic extensions. The vegetative nucleus has an average volume of 587μm^3. The sperm cells exhibit size dimorphism; the larger sperm cell has a volume of 141μm^3 and the smaller sperm cell has a volume of 86μm^3. This information on the MGU of *Asclepias tuberosa* will contribute to an understanding of the mechanism(s) underlying double-fertilization and self-incompatibility reactions in this species.

2. Flow Cytometric Characterization of Embryogenesis in Brassica napus Microspore Cultures

Derek Schulze and Peter Pauls, Crop Science U of Guelph

Microspore cultures of Brassica napus cv. Topas were analyzed flow cytometrically to characterize and isolate those microspores that are embryogenic. Embryogenesis in microspore cultures was induced by a high temperature treatment (30°C). Controls kept at 25°C did not produce embryos. Flow cytometric analyses were carried out at 24h intervals for 5 days and included light scatter and vitality measurements after staining with fluorescein diacetate. The results were correlated so that viable cells could be tracked. This allowed the identification and sorting of embryogenic cells. Subpopulations identified by flow cytometry were sorted and examined by light and epifluorescent microscopy. These analyses identified characteristic developmental pathways for each culture temperature. Low temperature microspores that remained viable after five days were enlarged, elliptical and had a dense cytoplasm. Five day old high temperature microspore cultures also had enlarged cells with a dense cytoplasm, but were spherical in shape. Thus, the large spherical microspores found in the high temperature induced cultures may be the embryogenic cells.

3. Bruni, Nadia C.[1], Jane P. Young[2] and Nancy G. Dengler[1]. [1]Department of Botany, University of Toronto. 25 Willcocks Street, Toronto, Canada M5S 3B2. [2]Faculty of Natural Resources and Environmental Studies, University of Northern British Columbia, [Prince George, B.C. V2N 4Z9]. - The developmental plasticity of Ranunculus flabellaris in response to aerial and aquatic environments.

Ranunculus flabellaris, an aquatic buttercup, exhibits heterophylly in morphology and internal anatomy in response to fluctuating water levels. Leaves developing underwater have elongate major lobes, epidermal cells are narrow and rectangular, and mesophyll cells are wide and rectangular. Stomata are absent. Water leaves are also thin, with a low percentage of intercellular space, and a lower mesophyll surface area than terrestrial leaves. The terrestrial habitat induces leaves with broad major lobes and isodiametric epidermal and mesophyll cells. These thicker leaves have a greater percentage of intercellular space, and a higher mesophyll cell surface area than aquatic leaves. Transfer of terrestrial plants to underwater conditions at successive stages of leaf development showed that the developmental pathway of these leaves has the potential to change morphologically and/or anatomically, depending on the transfer time. Multivariate analysis determined that area, lobe number, lobe length, dry weight, mesophyll surface area, and leaf thickness contributed the most to variation among the treatments. Our results show that these heterophyllous leaves can respond to environmental change at very late developmental stages.

4. GERRATH, JEAN M.[1,2] and RICHARD CÔTÉ[2] -- Orientation of cellulose fibers in pea tendrils. [1]Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614-0421, USA and [2]Department of Horticultural Science, University of Guelph, Guelph, ON N1G 2W1.

The surface features of tendrils and petioles of both conventional ("Improved Laxton's Progress") and semi-leafless ("Curly") peas were examined during their macroscopic development, using scanning electron microscopy. Leaflets of the conventional cultivar were also examined. There were no cultivar differences between the two types of tendrils. Both possess an adaxial groove that corresponds to the convex (outer) surface of the coil, and a distinctive tip. However, the most notable feature is the presence of cell surface ridges perpendicular to the long axis of the tendril. These become less prominent in an acropetal manner as the tendril elongates and coils, and may completely disappear on the convex surface once coiling is complete. The ridges are present to a lesser extent on the petioles, but are absent from the leaflets. Histological examination shows that these ridges are not part of the cuticle proper, but are part of the cellulosic cell wall. Thus, these ridges can provide a direct, non-intrusive marker for the manner in which pea tendrils elongate and coil.

5. Effect of Viral Infection on Cell Wall Integrity

Fauzia Siddiq, Sandra A. Kofalvi and Annette Nassuth.
Dept. of Botany, Univ. of Guelph, Guelph, Ontario, Canada, NIG 2W1.

Viral infection has profound effects on plant cell wall integrity. Our laboratory is interested in the effect of WSMV infection on monocot cell walls, especially on wheat cell walls (Type II cell walls). Microscopical studies have shown that walls from mesophyll cells in WSMV infected tissue (I) but not in healthy tissue (H) collapse after infection. The activity levels of enzymes which might be involved in this cell wall collapse were assayed. A higher xylanase activity was detected in extracts from I tissue compared to those of H tissue from 12 to 14 days after infection whereas no difference was found for 1,3:1,4-beta-D glucanases activity at any time. A plate assay has been developed in our laboratory to assay endo-xylanase activity. This plate assay suggests that two different isoforms of xylanases are present in wheat. We are currently working on separating these two isoforms by an activity gel assay.

6. EXAMINATION OF THE PHENOLIC COMPOUNDS IN CONCORDE AND FREDRICK WHEAT AND BARLEY SPP.

C.L. Miles and A.M. Zobel. Department of Chemistry, Trent University, Peterborough, Ontario. K9J 7B8

This study, provoked by the interest of agricultural crops, investigated the role of phenolic compounds in wheat. An initial investigation into the potential role of frost resistance was superseded by examining germination differences encountered between spring and winter varieties of wheat. The latter experienced a delay and often lack of germination in comparison to the spring variety. Current TLC results using water, acetic acid, and n-butanol (5:1:4) as a solvent have indicated a potential presence of quercetin, coumarin, or kaempferol within the winter wheat and possibly the same in smaller concentrations within the spring wheat. The barley and spring wheat samples experienced an increse in phenolic compounds for the first four days of stress (UV, low temperature) which then tappered off. The surface phenolic compounds on grains of both species are different.

7. RESPONSE OF FLAVONOIDS TO CADMIUM TREATMENT

B.F. Primeau and A.M. Zobel. Department of Chemistry,
Trent University. Peterborough, Ontario. K9H 3Y7

Flavonoids are natural occurring products which are produced in plants, they are usually accumulated in the vacuole. The anthocyanins which are part of this group, absorb different wavelengths of light and are responsible for the red colour of *Brassica oleracea*. Changes in flavonoid concentrations were observed in red cabbage seedlings, only 12 hours after being introduced to a 10umM and 100umM concentrated solutions of $Cd(NO_3)_2$. We postulate that flavonoids can react as antioxidants in both the vacuole and outside the cells.

8. REGULATION OF ANTHOCYANIN BIOSYNTHESIS IN PEA, *Pisum sativum*

Anne Uimari and Judith Strommer, Department of Molecular Biology and Genetics
University of Guelph, Guelph, Ont. N1G 2W1

Anthocyanins are red, blue and orange flavonoid pigments produced in epidermal plant cells and functioning in pollination, defence and resistance mechanisms. Anthocyanin biosynthesis, through the phenylpropanoid and flavonoid pathways, involves many structural and several regulatory genes. Cloned regulatory genes from maize and snapdragon can be classified into two groups: *myc*-like genes and *myb*-like genes, encoding transcription factors with similar DNA-binding and dimerization motifs as found in the animal *myc* and *myb* proto-oncogenes. In pea, *Pisum sativum*, two main anthocyanin biosynthesis regulatory loci, *a* and a_2, and several loci with quantitative effects have been identified. None of them has been cloned. We have taken two approaches in trying to understand the regulation of anthocyanin production in pea. We have studied the complementation of pea *a* and a_2 mutants by maize regulatory genes using particle bombardment. Our results indicate that the *myc*-like genes *Lc* and *R-S* are able to functionally complement both mutants. We have also attempted to clone *myb*- and *myc*-like genes from pea using PCR with degenerate primers. To date partial fragments representing two flower bud-expressed *myb*-like single copy genes have been cloned; we are attempting to determine the relation of these genes to the regulation of anthocyanin biosynthesis in pea.

9. THE EFFECTS OF UV RADIATION AND ACID SPRAY, ON THE PRODUCTION OF SOME SECONDARY METABOLITES IN *Daucus carota* AND *Ruta graveolens*.

C.Bates and A.M.Zobel. Department of Chemistry, Trent University, Peterborough, Ontario. K9J 7B8

The effects of certain bands of ultraviolet (UV) radiation as well as differing levels of acid spray were investigated on both *Daucus carota* and *Ruta graveolens*. The wave lengths that were used were 366nm, which is a natural wavelength that penetrates to the surface of the earth and 254nm which gets filtered out before it can reach the surface of the earth. Preliminary data suggests that with the introduction of the *Daucus carota* to the unfamiliar 254nm UV stress that production of both anthocyanins and flavonoids was increased.

Acid spray stress was the second focus of this investigation and variety of pHs were used starting at 0.5, 2.5, and finally 5.5. The purpose of using a solution as low as pH if 0.5 was in order severely damage the outer membranes on the surface of the leaf and thus lyse them. Once again preliminary results suggest that there is an increase in the production of the anthocyanins and flavonoids with regards to the *Daucus*. The investigations into the effects on the *Ruta* are still ongoing for both stress groups. From these investigations the following secondary metabolites are hoped to be isolated and identified. These compounds will be quercetin, kaempferol, rutin, coumarin, umbelliferone, and peucedanin.

10. **THE BIOCHEMICAL CHANGES IN PLANT CELLS DUE TO TOXIC CONCENTRATIONS OF SILVER**

Teresa Switzer and Alicja Zobel, Department of Chemistry, Trent University, Peterborough, Ontario K9J 7B8.

There have been very high concentrations of silver found in soil and rock samples from Peru; often as high as 300 grams per tonne. As well, in particular areas of Lake Ontario it was found that the concentration of silver was so high that it was possible to develop photographic films in it. This high concentration of silver must affect plants. We found that 300 ppm silver nitrate ($AgNO_3$) affects Tradescantia plants, which are native to warm climates. The silver was absorbed by the plants and transported to the top of the shoot in various proportions. The silver caused shrinkage and blackening of tissues in the plant shoots, with a very distinctive barrier being formed between the black tissue and the green tissue on the stems. Such barriers contained drastic changes in concentrations of accumulated silver ions.

11. **The Influence of Tea and Echinacea on the Chemistry of the Mitochondria Treated with Olive Oil**

Foo-Lim Yeh and Alicja M. Zobel
Dept. of Biochemistry, Trent University, Peterborough, Ontario, Canada K9J 7B8

The effects of green tea and the extract of Echinacea on oxygen consumption of mitochondria were measured. Assays were performed on isolated beef liver mitochondria treated with ordinary cooking olive oil. Oxygen consumption was measured by means of a Gilson Differential Respirometer. It was found that both green tea and Echinacea reversed the effects of olive oil on mitochondria by stimulating respiration. Echinacea stimulated oxygen consumption at various concentrations tested, while this was true for green tea to a certain extent. At low concentrations, green tea neither stimulated nor reduced respiration. These results suggest that both green tea and Echinacea have the ability to stimulate components of the respiratory chain and oxidative phosphorylation.

12. Cellular Effects of Roundup®

C.A.Swackhammer and A.M.Zobel Department of Chemistry, Trent University, Peterborough, Ont. K9J 7B8

The herbicide Roundup® is one of the most extensively used herbicides. It has a very high commercial and private use; because it is widely used many animal species are also exposed to its effects. Many papers have been written that examine this herbicide's effects on varying plant metabolic pathways but few deal with pathways common to plants and animals. The examination of garlic root cells illustrated that Roundup® does not cause chromosomal abberations. The postincubation cells showed unusually large nucleoli and only cells in interphase. This suggests that the cell cycle has stopped preventing the cells to enter another mitotic phase.

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